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HYDROQUINONE IS NOT A PHAGOSTIMULANT FOR THE FORMOSAN SUBTERRANEAN TERMITE

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Abstract—It has been suggested that hydroquinone found in the labial glands of a number of termite species acts as a primary phagostimulating factor. We tested hydroquinone as a phagostimulant using workers from three colonies of the Formosan subterranean termite, *Coptotermes formosanus*, under both laboratory and field conditions. Hydroquinone at concentrations ranging from ca. 0.002–20.0 ng/cm^2 did not increase visitation by *C. formosanus* workers to treated over control filter papers, and was actually repellent at a 20 ng/cm^2 dose. No phagostimulant response to hydroquinone was observed in two colonies. In the third, there was a significant increase in feeding on filter paper treated with a 2 ng/cm^2 dose, but was significantly lower at a 20 ng/cm^2 dose. Furthermore, sand treated with a gradient of hydroquinone, did not evoke increased tunneling activity compared with controls. GC-MS analysis of *C. formosanus* workers indicated that hydroquinone was present at an average of 41 pg/worker. It was also determined that within one week about 11% hydroquinone in aqueous solution oxidized to 1,4-benzoquinone. Our findings indicate that hydroquinone alone does not act as a phagostimulant but instead may act as a repellent at higher concentrations. The attractant/arrestant of the Formosan termite may have multiple components of which hydroquinone, at low doses, could be one.

Key Words—Formosan subterranean termite, *Coptotermes formosanus*, hydroquinone, phagostimulant.

INTRODUCTION

Termite workers foraging for food use trail pheromones to guide other members of their caste to foraging sites (Pasteels and Bordereau, 1998). Once a suitable food source is located, the termites tend to stay and feed at that site.

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Reticulitermes santonensis and *Schedorhinotermes lamanianus*, have a highly polar, heat-resistant, and nonvolatile component of their labial glands, which when released onto food promoted aggregation and feeding, and was designated a phagostimulating pheromone (Kaib and Ziesmann, 1992; Reinhard and Kaib, 1995, 2001a,b; Reinhard et al., 1997). Using feeding bioassays conducted with *R. santonensis* and *S. lamanianus*, Reinhard and Kaib (2001b) reported feeding-stimulating activity in extracts of labial glands from 11 termite species from five families including one from the Formosan subterranean termite, *Coptotermes formosanus*. Based on these observations, they suggested that such a general feeding-stimulating signal might have evolved in many termite species. The feeding-stimulating component in the labial gland extract was subsequently identified as 1,4-dihydroxybenzene[2] or hydroquinone and varied in concentration from 0.02 ng in *Kalotermes flavicollis* to 10.0 ng in *Mastotermes darwiniensis*, with 0.03 ng in *C. formosanus* (Reinhard et al., 2002a). Doses of 5 and 10 ng hydroquinone applied to 2.5 cm^2 filter paper disks resulted in a phagostimulating effect in *M. darwiniensis*, as did a concentration of 100 ng/cm^2 in field tests carried out in Malaysia with *Coptotermes curvignathus* (Reinhard et al., 2002a).

The Formosan subterranean termite was introduced into the United States during the middle of the 20th century and has since become a serious pest of homes and living trees in several southern states. With increasing restrictions on the use of conventional insecticides, there is need to find environmentally safe technologies to manage this pest. Baiting was suggested a long time ago (Esenther and Beal, 1978) as a means of controlling infestations, and with the introduction of chitin synthesis inhibitors, it has become the treatment of choice (Su, 1994). However, a major constraint in the use of this technology is the failure of the baits to attract termites. Several additives have been used in bait matrices (primarily cellulose containing materials) to make these attractive (Chen and Henderson, 1996; Rojas and Morales-Ramos, 2001). Reinhard et al. (2002b) suggested the use of hydroquinone in bait systems for termite management as both laboratory and field tests, indicated increased feeding by *M. darwiniensis* and *C. acinaciformis* in response to the treatment. In addition, hydroquinone was also reported to act as an attractant over a short distance.

We have also observed that a substrate on which termites have been feeding for some time acts as an attractant/arrestant for conspecific individuals (unpublished information). Therefore, we undertook this study to evaluate hydroquinone as an attractant-arrestant phagostimulant for *C. formosanus* both under laboratory and field conditions.

[2]Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

METHODS AND MATERIALS

Insects. Three field colonies of *C. formosanus* from existing bucket traps were selected. Colonies S-67 and S-6B were from the grounds of Southern Regional Research Center and U-70 from the University of New Orleans (UNO), Lakefront campus, both in New Orleans, LA USA. After collection, the termites along with fresh spruce wood pieces were placed in plastic boxes and kept in an incubator maintained at $28 \pm 1^{\circ}$C, $80 \pm 5\%$ RH and constant darkness.

Attraction/Phagostimulation Assay. A thin layer of sand (15 g) was poured into individual plastic Petri dishes (90×13 mm) and moistened with 2.5 ml distilled water. Two, 2.5 cm diam Whatman #1 filter papers were weighed and each lightly marked with a pencil either as T or C for treated or control, respectively. Hydroquinone (Aldrich, Milwaukee, WI) was dissolved in distilled water to obtain concentrations of 0.01, 0.1, 1.0, 10.0, and 100.0 ng/50 μl (hydroquinone was readily soluble at these concentrations). The filter paper disks were treated either with 10 μl of one of the hydroquinone solutions or water and placed at opposite ends of the Petri dish. Twenty workers were released into each dish, and the dishes were placed under a CCD camera (Emcal Scientific, Poway, CA) under low intensity red light. Distribution of the workers was recorded every 15 min for 3 hr, using a Panasonic AG-6740 time-lapse video recorder. After 3 hr, an additional 80 workers were released into each Petri dish (to obtain greater feeding) and placed in the incubator. After 24 hr, the filter papers were removed, washed, oven dried, and weighed. The assay was repeated six times for workers from each of the three colonies at all five concentrations of hydroquinone. Average consumption was determined for each treatment and each colony.

Tunneling Bioassay. A plastic container ($75 \times 75 \times 30$ mm) was connected to a glass T-tube via a piece of Tygon tubing inserted into a hole drilled into the side (Figure 1A). Each free end of the T-tube was connected with a Tygon connector to a 15 cm tube (plastic pipette, 5 mm ID) filled with fine sand. To one tube, a hydroquinone solution was added with a glass syringe through two small holes 5 cm apart (Figure 1B) at the proximal end to produce a gradient of 0.1 to 1.0 to 10.0 ng in 230 μl water, with the lowest concentration being closest to the T-tube. In the other, the sand was treated with distilled water and acted as control. Both tubes were covered with aluminum foil. Termite workers (100) were released into the square container, and tunneling activity through the sand in plastic tubes was monitored every 15 min for 2 hr and then every hour for the next 4 hr. The assay was repeated four times for each of the three colonies.

Field Test. In an initial test, paper towels treated with 125 ng/cm^2 hydroquinone, were strongly repellant, so in the actual field trials a weaker solution was used. In this test 20×20 cm paper towels (Bounty®, Procter and Gamble, Cincinnati, OH) were weighed and treated with either 800 ng hydroquinone (2 ng/cm^2) in 2.6 ml distilled water or just water. The towels were folded, enclosed

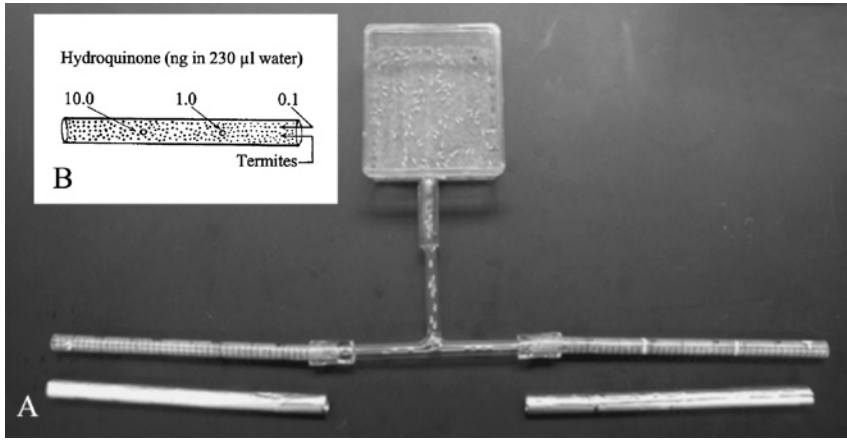

FIG. 1. Setup for tunneling assay. A. The square plastic container with the termites is connected to two tubes through a T-tube. Two aluminum foil covers were slipped over the tubes to exclude light. B. Each tube is filled with sand treated either with water or with hydroquinone in an increasing gradient.

in Tygon tubing (10 cm × 2.2 cm ID) and placed in three different bucket traps (three treated and three control tubes/trap) on the UNO campus after removing the wood. After 72 hr, the tubes and enclosed towels were removed. Each towel was carefully washed, oven dried, and weighed. Average consumption was calculated. The test was replicated four times for each of the three colonies.

Determination of Endogenous Hydroquinone. Freshly coll Taluminar

followed by a ramp to 160°C at 3°C/min. Quantitation was based on *m*/*z* 239 using a standard curve of TMS-derivatized hydroquinone.

Hydroquinone Oxidation Test. Hydroquinone (30 μg in 50 μl distilled water) was applied to 2.5 cm diam filter paper disks (Whatman #1) placed in three 100 × 15 mm plastic Petri dishes (5/dish) in addition to one dish containing water treated control paper disks, and stored at room temperature and in darkness. Five disks were removed after 0, 1, or 4 days and individually extracted with 3 × 100 μl ether. Extracts (1 μl) were tested by GC-MS as described above but with a temperature program from 30°C with a 1 min hold to 210°C at 10°C/min. Quantitation was done using single ion monitor (SIM) at *m*/*z* 110 and 108 for hydroquinone and benzoquinone, respectively.

Data Analysis. All the data were analyzed using an unpaired *t*-test, Graph Pad Prism version 4.0 (Graph Pad Software Inc. 2003, San Diego, CA).

RESULTS

In the choice test, the workers showed no significant preference for either treated or control disks at hydroquinone concentrations between 0.002 and 2.0 ng/cm^2, but in all three colonies a concentration of 20 ng/cm^2 had a significant repellant effect (Table 1). In two of the three colonies, there was no evidence that hydroquinone affected feeding behavior as the quantities of treated and control paper consumed did not differ significantly. In the third colony, there was evidence that hydroquinone acted as a phagostimulant at a concentration of 2.0 ng/cm^2 but was a feeding deterrent at 20 ng/cm^2 (Table 2).

In the tunneling assay, the distance tunneled in treated and control arms, as a function of time, did not differ significantly in any of the colonies tested (Table 3). For both control and treated tubes, the average distance tunneled in 6 hr was about

TABLE 1. PERCENT DISTRIBUTION OF *C. formosanus* WORKERS FROM THREE COLONIES ON HYDROQUINONE VS. CONTROL FILTER PAPER DISKS[a]

Treatment dose (ng/cm^2)	S-67		U-70		S-6B	
	C	T	C	T	C	T
0.002	6.3 ± 1.7	5.3 ± 0.9	6.7 ± 2.8	3.6 ± 1.7	5.2 ± 1.8	6.8 ± 2.6
0.02	3.3 ± 0.5	6.3 ± 1.8	4.6 ± 2.1	3.3 ± 0.4	3.9 ± 1.3	8.7 ± 2.5
0.2	8.4 ± 1.6	5.9 ± 1.2	9.6 ± 5.1	6.3 ± 3.6	10.0 ± 2.6	6.5 ± 2.7
2.0	6.6 ± 0.8	5.4 ± 1.8	8.1 ± 3.6	3.7 ± 1.4	4.7 ± 1.0	4.7 ± 1.3
20.0	11.8 ± 2.1	6.2 ± 1.0*	11.3± 3.0	1.7 ± 0.3**	11.2 ± 2.5	2.6 ± 0.3**

[a]Choice test. Filter paper disk (2.5 cm diam) treated with hydroquinone (T) or water (C). Location of the number of workers out of 20 that were on either of the filter paper disks, recorded every 15 min for 3 hr. Average % distribution ±SE, $N = 6$ for each of the 3 colonies. Means significantly difference at $P < 0.05$(*) and $P < 0.01$(**).

TABLE 2. AVERAGE CONSUMPTION (mg) OF CONTROL VS. HYDROQUINONE TREATED FILTER PAPERS BY WORKERS OF *C. formosanus* FROM THREE COLONIES[a]

Treatment dose (ng/cm^2)	S-67		U-70		S-6B	
	C	T	C	T	C	T
0.002	5.8 ± 1.0	3.4 ± 0.6	3.6 ± 0.4	4.2 ± 0.7	6.3 ± 0.9	5.9 ± 0.6
0.02	4.7 ± 0.9	5.2 ± 0.3	3.9 ± 0.7	4.6 ± 1.1	7.0 ± 0.7	6.2 ± 0.6
0.2	4.4 ± 0.9	3.8 ± 0.6	4.1 ± 0.4	2.9 ± 0.8	7.9 ± 1.4	7.6 ± 1.0
2.0	3.0 ± 0.3	5.4 ± 0.8*	2.4 ± 0.6	3.7 ± 0.8	8.5 ± 1.3	6.1 ± 1.2
20.0	5.7 ± 0.9	3.2 ± 0.5*	3.3 ± 1.3	3.0 ± 0.8	6.3 ± 0.4	6.2 ± 0.4

[a] Choice test with filter papers (2.5 cm diam) treated with hydroquinone (T) and water (C) presented to 100 workers over a period of 24 hr. Consumption is the average ±SE of 6 replicates for each colony. Means significantly different at $P < 0.05$(*).

79 mm. After 24 hr, workers of U-70 and S-6B had tunneled the entire length of the tube in all cases and come out at the open end (data not presented). Workers of colony S-67 had also tunneled through the tube but did not come out.

In the initial field test, hydroquinone at 125 ng/cm^2 of paper toweling strongly repelled the termites resulting in their abandoning the traps. In the subsequent test, there was no significant difference in consumption of the paper towels between the treated (2 ng/cm^2) and control groups for any of the three colonies tested (Table 4).

Hydroquinone was detected in the whole body extracts of all workers for the three test colonies (Figure 2). Levels ranged from 1–159 pg/worker across all samples with a colony average of 41 ± 37 pg/worker. Hydroquinone was fairly stable over the period used in the bioassays. After one day under conditions similar to assays with termites, recovery of hydroquinone was similar to 0 day, with no increase in the oxidation product (1,4-benzoquinone). Subsequently, the stability of hydroquinone was tested by observing the amount of 1,4-benzoquinone by GC-MS obtained from treated paper disks under conditions similar to the bioassay. The benzoquinone/hydroquinone ratio, after exposure to atmospheric conditions

TABLE 3. AVERAGE DISTANCE (mm) TUNNELED BY *C. formosanus* WORKERS FROM THREE COLONIES THROUGH SAND TREATED WITH A CONCENTRATION GRADIENT OF HYDROQUINONE (C) VS. CONTROL SAND (T)[a]

Time hours	S-67		U-70		S-6B	
	C	T	C	T	C	T
1	12.2 ± 2.6	14.2 ± 3.2	13.0 ± 1.8	11.7 ± 1.7	10.7 ± 2.2	15.0 ± 1.2
2	25.2 ± 3.4	33.2 ± 1.9	27.7 ± 2.5	29.2 ± 1.3	28.0 ± 1.3	28.3 ± 2.6
3	36.7 ± 3.3	43.7 ± 3.6	47.7 ± 2.5	44.7 ± 3.1	37.0 ± 4.8	41.0 ± 3.7
6	69.7 ± 5.9	69.5 ± 3.0	85.2 ± 4.3	80.7 ± 3.9	80.7 ± 5.2	86.7 ± 3.1

[a] Total length of the tube with sand was 150 mm. Average distance ±SE, $N = 4$.

TABLE 4. AVERAGE WEIGHT OF CONTROL AND HYDROQUINONE TREATED PAPER TOWELS CONSUMED BY *C. formosanus*, IN FIELD TRAPS OVER A PERIOD OF THREE DAYS[a]

Colony	Control	Treated
U-70	695.7 ± 574.4	128.3 ± 49.5
U-101	1471.0 ± 376.6	1607.0 ± 279.7
Undesignated	1450.0 ± 156.9	1572.0 ± 382.9

Note. Consumption of hydroquinone treated (2 ng/cm^2) and control paper towels placed in traps for three days. Average mg ± SD, $N = 4$.

for up to 4 days, had not significantly changed (data not shown) although the total recovery had decreased.

DISCUSSION

Through a series of publications it was suggested that labial glands of a number of termite species contain a phagostimulating factor (Reinhard and Kaib, 1995; 2001a,b; Reinhard et al., 1997). The factor was subsequently identified as

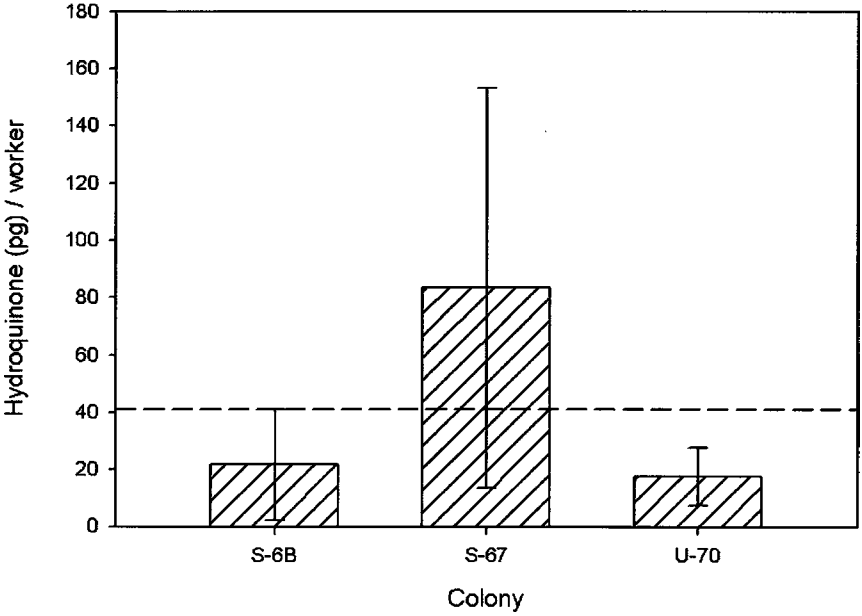

FIG. 2. Amounts of hydroquinone (mean ± SD, $N = 3$) from whole body extraction of three colonies of *C. formosanus* workers. Broken line indicates colony average.

hydroquinone (Reinhard et al., 2002a). It was also shown that hydroquinone was present in the labial gland extract from 15 termite species including *C. formosanus*. They further reported that the compound significantly stimulated feeding by *M. darwiniensis*, on filter papers treated at 2 and 4 ng/cm^2. Consequently, we tested hydroquinone as an attractant/phagostimulant for *C. formosanus*, in comparable tests carried out both in the laboratory and the field. Hydroquinone at concentrations ranging from 0.002–20 ng/cm^2 did not evoke preferred visitation by *C. formosanus* workers to treated filter paper over control paper. On the other hand, the compound was repellent at the 20 ng dose. In rare instances, we observed an initial attraction to hydroquinone treated filter papers with the workers quickly dispersing uniformly. Similarly, no significant phagostimulant response was observed except for workers from one of the test colonies and at only the 2.0 ng dose. However, what is puzzling is that in our experiments, hydroquinone acted as a strong repellent at higher concentrations. Recently, it was reported that in choice tests with *C. formosanus*, hydroquinone did not induce increased consumption at lower doses, whereas at higher doses (1 ng/mg filter paper) the compound acted as a feeding deterrent (Cornelius, 2003). Sand treated with a gradient of hydroquinone, did not evoke increased tunneling activity. In the field test, using a dose comparable to the one used by Reinhard et al. (2002a) for *C. curvignathus*, we found that the termites quickly abandoned the traps. When we used a concentration that had been shown effective for *M. darwiniensis* in the laboratory, there was no difference in feeding between control and treated paper towels.

Analysis of *C. formosanus* workers indicated that hydroquinone indeed was present at an average of 41 pg/worker (colony range: 20–80 pg). This is in conformity with the findings of Reinhard et al. (2002a). They reported an average of 30 pg hydroquinone per labial gland of *C. formosanus*. The stability of hydroquinone in aqueous solution was tested under conditions much more rigorous than the assay, and only a small increase in the amount of oxidation product (1,4-benzoquinone) was observed.

Our results indicate that response of termites to hydroquinone may be species or even colony specific. We have observed that extracts of substrate on which termites were allowed to feed for some time is active as an attractant/arrestant (Raina: unpublished results). The identification of active component(s) in the extract is in progress. It may well be multi-component, of which hydroquinone at very low concentrations could be one of the components. It is also possible that the active factor may be derived from the interaction of termite excretions, primarily from labial glands, on the cellulose-based substrate. Identification of such an attractant will be valuable for use in baits to increase their efficiency.

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